A ring trial for testing the comparability of the laboratory results of three commercial Salmonella antibody ELISA tests in Germany, Denmark and The Netherlands

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Abstract

Three commercial and one non-commercial ELISA test kits for detecting Salmonella antibodies in meat juice of pigs were tested in an international ring test. All test kits proved to produce highly comparable results. The result has relevance for the upcoming Salmonella control strategy in the EU, if the national Salmonella reduction measures are planned to be based on a serological risk categorisation of pig herds.

Introduction

Since 2002, a serological Salmonella monitoring programme has been carried out in all German finishing pig herds that participate in the "QS-System", a voluntary national quality management system approving the correctness of the production procedures for food resulting in the control stamp "QS". This monitoring aims at categorising the participating herds (40% of all German herds representing 75% of the German pork production) according to the risk of introducing Salmonella into the pork chain via infected slaughter pigs into three categories (I = low, II = middle, III = high). The classification into the categories is calculated quarterly based on the percentage of Salmonella antibody positive meat juice samples within a random sample of 60 per year for each farm. All data generated within the monitoring are entered into the central database Qualiproof® (Qualitype AG, Dresden), which provides automatically the categorisation every quarter of a year and suggest the daily sample size at slaughter for every herd participating (ANONYMOUS, 2007).
For the acceptance of the results obtained by using three commercial and additionally "QS-approved" ELISA test systems in "QS-approved" laboratories, it is extremely important to make sure that the results of all three tests in all laboratories are comparable. Therefore, every laboratory that wants to serve the QS-system has to take part in the yearly ring trial for maintaining their "QS-approval" valid.

Material and methods

From a multitude of pre-tested single meat juice samples, forty mixed meat juice samples à 50 ml were pooled in a way that 10 of these samples were adjusted to be highly positive (> 80 OD%), 10 to be highly negative (< 10 OD%), and 20 were adjusted to have OD% values around the cut-off value of 40 OD% (30 – 50 OD%).
These pooled meat juice samples were aliquoted into 1640 single test samples. These test samples were enumerated using a random generator, lyophilised and sent to 43 laboratories (4 Dutch, 1 Danish and 38 German labs) taking part in the 2006 ring trial. The samples were, of course, absolutely unknown to all laboratories.
The lyophilisation was chosen to minimise any thinkable influence of different treatment of the samples before using them in the ring trial such as failures in the freezing/cooling chain, multiple freezing and thawing procedures of the same sample and the like. Every laboratory was asked to apply its routinely used method and test system according to the test producer's instructions. The

three commercial and "QS-approved" tests that were included into the ring trial used in the German laboratories and in the Dutch laboratories were: SALMOTYPE® Pig Screen (Labor Diagnostik Leipzig, Leipzig), HerdCheck® (IDEXX), and Enterisol® (Boehringer Ingelheim). The Danish laboratory used its own, non-commercial, but well established "Danish mixed ELISA".

Results

Two laboratories were excluded from the evaluation of the ring trial, since their results were completely non-congruent with the expected outcome. Both laboratories (No. 13 and 16) had only applied for the QS-approval and had used the test kits for the first time - they did not get the QS-approval.
All other participating laboratories showed a satisfying degree of congruity compared to the results of the Danish laboratory (the results of which were used as reference values). All laboratories detected the highly positive samples as "high positive" and all highly negative samples as "high negative" (see Figure 1a and 1b).

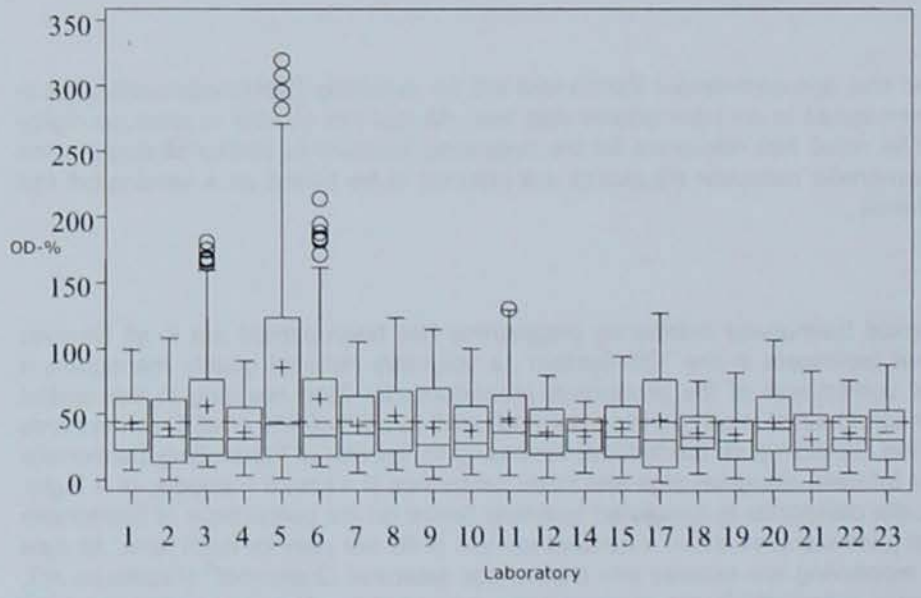

Figure 1a. Graphical demonstration of the results of all laboratories in OD% for all samples

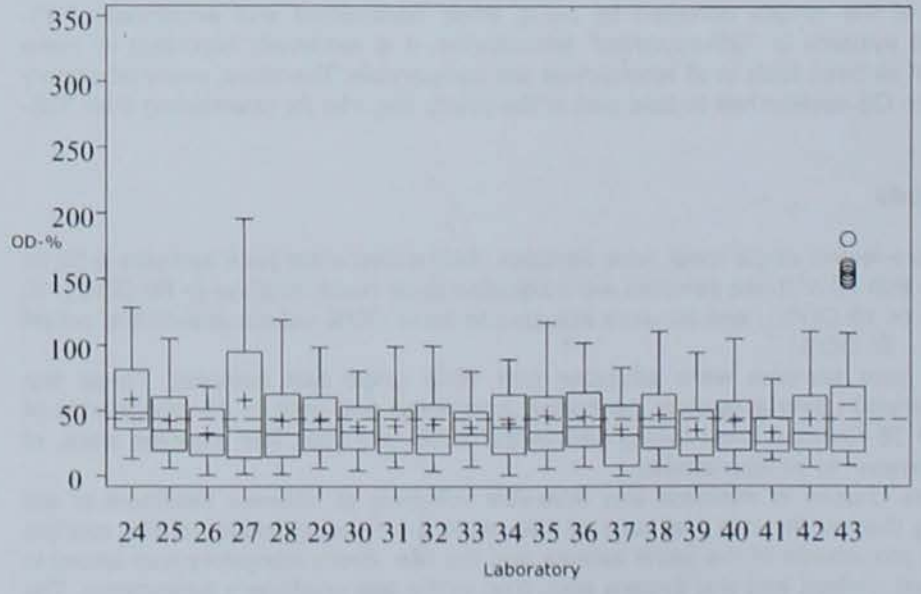

Figure 1b. Graphical demonstration of the results of all laboratories in OD% for all samples

The results of the accuracy of the tests and the laboratories in terms of assigning a sample to "positive" or "negative" can be seen in Figure 2.

	Testkit: PigScreen																										Testkit: IDEXX											Testkit: Enterisol			Dan. mixed ELISA
Sample	L01	L04	L05	L07	L08	L10	L11	L15	L18	L19	L22	L23	L24	L25	L28	L29	L30	L31	L32	L33	L35	L36	L38	L39	L40	L42	L02	L09	L12	L14	L20	L21	L26	L27	L34	L37	L41	L03	L06	L43	L17
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	1	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0
40	0	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	1	0	0
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Figure 2. Dichtomised results of all samples and laboratories sorted according the median of the OD% values over all laboratories (0 = "negative", < 30 OD%; 1 = "around the cut-off", 30 – 50 OD%; 2 = "positive", > 50 OD%; L = Laboratory)

The ring test results show that there are some differences between the tests, but again mainly in the "very high positive" samples. However, if the assignment of the samples to "positive" and "negative" is taken into consideration, only samples "around the cut-off" differ from test to test (see Figure 3)

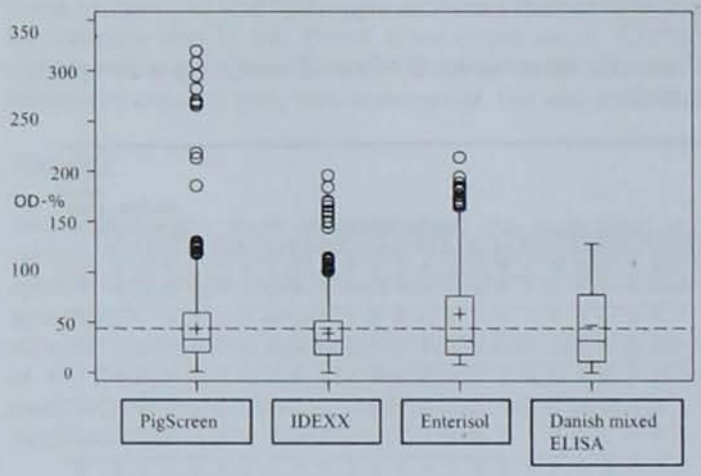

Figure 3. Graphical demonstration of the results sorted by test kit

Discussion and conclusion

There are huge differences in the positive values between the laboratories. These differences are due to the fact that some laboratories capped the positive values at 100 OD%, whereas others did not (the highest positive value measured was 319.7 OD%).
Since, however, the categorisation of the monitoring programme is based on the percentage of Salmonella "positive" animals in the random sample of 60, only the accuracy of the dichtomised decision ("positive" or "negative") is of importance for the accuracy of the monitoring. The fact that the group of "around the cut-off" samples show a lower degree of congruity, is "natural", since a sample measured with 39.9 OD% in one laboratory (or with one test) is "negative", and measured with 40.1 OD% in another laboratory (or another test) is "positive", although both laboratories (or tests) were very accurate. However, taking into consideration that only 10% to 15% of the samples in the field are around this cut-off (and not 50% as in the artificial test sample collection), and that the categorisation is always based on 60 samples, it becomes obvious that the few samples around the cut-off value in the 60 sample do not really influence the categorisation.
Summarising the results of the presented ring trial it can be said: the tested three commercial Salmonella antibody ELISA tests are highly comparable with the original Danish mixed ELISA, they are robust in terms of their repeatability and usability in various laboratories. These two characteristics of the tested tests is very important in the light of the EU Directive 99/2003/EC and the EU Regulation (EC) 2160/2003, since the harmonisation of Salmonella antibody ELISA tests for the categorisation of pig herds according to their risk of introducing Salmonella into the food chain is a prerequisite for the comparability of the Salmonella surveillance and reduction programmes in the EU member states (VAN DER HEIJDEN, 2001; VAN DER WOLF et al., 2001).

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